The influences of hyperbaric oxygen therapy with a lower pressure and oxygen concentration than previous methods on physiological mechanisms in dogs

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ABSTRACT. Recently, hyperbaric oxygen therapy with a lower pressure and oxygen concentration (L-HBOT) than previous methods has been used for dogs in Japan; however, the influences of L-HBOT on dogs have not been clarified. To verify the influences of L-HBOT on physiological mechanism in dogs, we investigated blood gas parameters, glutathione peroxidase (GPx) activity, heart rate variability, stress-related hormones and skin conductance (SC) in 4 clinically normal beagle dogs with catheters in their carotid arteries and jugular veins when they were quiet, after running, after receiving L-HBOT (30% oxygen concentration, 1.3 atmospheres absolute, 30 min) or after not receiving L-HBOT. The results showed there were no changes in blood gas parameters, heart rate variability and catecholamine levels after L-HBOT. GPx activity was significantly higher, and the SC and cortisol level were lower in dogs that received L-HBOT than those when they were quiet. These results suggested that L-HBOT may have a small influence on oxygenation dynamics, activate antioxidant enzymes such as GPx, restrain autonomic nervous activity and control the balance between oxidation and antioxidation inside the body.
KEY WORDS: autonomic nervous system, blood gas parameter, canine, glutathione peroxidase, hyperbaric oxygen therapy

Hyperbaric oxygen therapy (HBOT) for human beings involves the inhalation of 100% oxygen in chambers pressurized at 2.0 to 2.5 atmospheres absolute (ATA) [41]. It increases the dissolved oxygen content to above physiological levels, according to three gas laws [12, 20]. Briefly, for a body of ideal gas at a constant temperature, the volume is inversely proportional to the pressure (Boyle's law). The solubility of a gas is proportional to the pressure of the gas in equilibrium with the liquid (Henry's law). The diffusion of a gas is proportional to the gas concentration gradient (Fick's law). According to these laws, HBOT induces some physiologic effects [13], such as gas bubble reduction [25, 45], improved oxygenation [48, 53], vasoconstriction [37], antimicrobial activity [31] and angiogenesis [33, 52].

However, some reports have indicated that HBOT with a high pressure and high oxygen concentration (H-HBOT) may induce barotrauma [4] or oxygen toxicity [32, 42]. Recently, HBOT with a lower pressure (1.2 to 1.3 ATA) and lower oxygen concentration (approximately 30%) (L-HBOT) than previous H-HBOT methods have been used not only for medical but also for personal use [12]. The chambers for L-HBOT are considered to cost less and to result in fewer complications than those for H-HBOT. For these reasons, L-HBOT has been used in veterinary medicine. L-

HBOT has been mainly used to maintain body homeostasis for animals that have perioperative stress in some veterinary clinics. Although there are some reports verifying the influences of HBOT in animals [8, 19, 49], these reports showed the influences of H-HBOT in animals. In contrast, no study has yet been carried out to verify the effects of L-HBOT. Therefore, in this report, we present the physiologic influences of L-HBOT in dogs.

In previous studies, H-HBOT influenced oxygenation dynamics [50], oxidative stress [3, 6, 15, 16] and the autonomic nervous system [1, 18, 39, 44]. However, the results of these studies varied and cannot to be said to apply to L-HBOT. Therefore, to investigate the influences of L-HBOT on these physiological mechanisms, we investigated the following measurements: blood gas parameters, which show the oxygen concentration in the blood, such as the partial pressure of arterial oxygen (PaO_2), arterial oxygen saturation (SaO_2), arterial oxygen content (CaO_2), arterial blood pH and partial pressure of carbon dioxide ($PaCO_2$), as an evaluation of oxygenation dynamics; glutathione peroxidase (GPx) activity, which is one of the most important antioxidant enzymes known to metabolize hydrogen peroxide and lipid hydroperoxides induced by reactive oxygen species (ROS), as an evaluation of oxidative stress; and heart rate variability at low-frequency/high-frequency power (LF/HF) and RR-intervals (RRI), cortisol, adrenaline and noradrenaline levels and skin conductance (SC) as an evaluation of autonomic nervous activity.

MATERIALS AND METHODS

Animals: Four beagle dogs (2 males and 2 females, 2 years old, weighing 9.9 to 11.3 kg) were included in this study. The dogs had no evidence of disease based on their

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histories and a clinical examination in which a blood sample had been taken as part of a routine health check. They were accustomed to being restrained, having blood samples drawn and taking medications. They had never been included in any study examinations before the present study. They were housed in individual cages, in which the temperature was maintained at $23 \pm 1°C$ and kept under a 12:12-hr light/dark cycle. The dogs were fed twice a day, at 10:00 and 16:00, and water was available freely. The protocols in this study were approved by the Animal Care and Use Committee of Osaka Prefecture University.

Blood sampling catheter placement: In order to precisely obtain blood samples, we placed catheters in the carotid arteries and jugular veins of the dogs. Seven days before the start of the study protocol, all dogs were preanesthetized with 0.05 mg/kg atropine sulfate hydrate subcutaneously, 0.25 mg/kg diazepam intravenously and 0.1 mg/kg butorphanol tartrate intravenously. The dogs were then injected with propofol intravenously and anesthetized with isoflurane at a 2.0% concentration in expiration via an endotracheal tube. The depth of anesthesia was monitored according to the AAHA anesthesia guidelines for dogs and cats [5], and an adequate anesthesia level was maintained for the operations. All dogs were injected with 30 mg/kg cefazolin subcutaneously as an antibiotic during the operations. The catheter (Arrow central venous catheterization set, single lumen, 18 Ga × 8", 20 cm, Teleflex Medical Japan Ltd., Tokyo, Japan) was placed in the left carotid artery and jugular vein according to a general method. Briefly, the left external carotid artery and jugular vein were surgically exposed. Two silk threads were placed around the artery. The artery was incised transversely with a number 11 scalpel blade and then dilated with a blunt instrument. A catheter is passed into the artery to the level of common carotid artery. The proximal thread was tightened around the catheter and then tied to the catheter to secure it. The vein was treated in the same way [36]. The catheters were flushed with saline containing 4.0 U/ml heparin sodium every 5 hr at night and during the day until they were removed to prevent of embolism [23]. All dogs were prescribed an antibiotic, 25 mg/kg cephalexin, to be taken orally twice a day from catheter placement to 7 days after catheter removal. During the 7 days from catheter placement to the start of the study, the dogs were acclimated to the catheters, the hyperbaric chamber and other apparatus used for this study. We removed the catheters after we had completed this study.

Study protocol: Seven days after catheter placement, we studied the 4 dogs in a crossover trial. We did all studies during the morning to exclude the effects of daily fluctuation. (1) When the dogs were quiet, we collected blood samples and measured parameters. (2) Next, the dogs ran on a treadmill (Corpo Motor Walker CP4000, S. N. T. Co., Ltd., Kanazawa, Japan) at 2.0 m/sec for 10 min to induce physiological stress. After running, we collected blood samples and measured parameters. Then, the dogs were placed randomly (3) in a normal cage for 30 min (non L-HBOT) or (4) in a hyperbaric chamber with 30% oxygen at 1.3 ATA for 30 min (L-HBOT). After these treatments, we collected
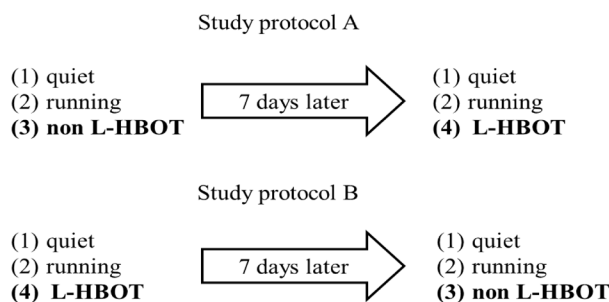

Fig. 1.   Study protocol. Seven days after catheter placement, we studied the 4 dogs in a crossover trial (1) when the dogs were quiet, (2) after running, (3) after non L-HBOT and (4) after L-HBOT. Briefly, a dog underwent one study protocol and then underwent the other 7 days later.

blood samples and measured parameters again. All dogs underwent treatment sets consisting of (1), (2) and (3) and (1), (2) and (4) randomly. Briefly, a dog underwent one treatment set and then underwent the other set 7 days later (Fig. 1). All dogs were subjected to general blood tests at every trial to exclude anemia or infections.

Hyperbaric oxygen chamber: We used a hyperbaric oxygen chamber for animals (O_2 Support-01, LiveAid, Co., Ltd., Kanazawa, Japan). The chamber was clear and had sufficient space for the dogs to walk around (870 mm length × 780 mm width × 790 mm height). Air was instilled into the chamber through an air compressor, which condensed the oxygen in the air to a 30% concentration using the pressure swing adsorption method. This method uses zeolite as an absorbent to absorb nitrogen and condense the oxygen in the air. The chamber was pressurized and maintained at approximately 1.3 ATA. To depressurize the chamber, we cut off the power of the pressure device and exhausted the pressurized air. Condensed air continued to be still instilled until the pressure decreased to 1.0 ATA.

Blood gas monitoring: PaO_2, SaO_2, pH and $PaCO_2$ were measured with a blood gas monitor (i-STAT 300F, Fuso Pharmaceutical Industries Ltd., Osaka, Japan). Approximately 0.5 ml arterial blood was collected from the catheter and used for measuring PaO_2 and SaO_2. CaO_2 was calculated using the following equation [10]:
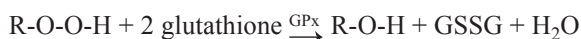
$$CaO_2 \ (ml/dl) = SaO_2 \ (\%)/100 \times \text{hemoglobin concentration in arterial blood} \ (g/dl) \times 1.39 + 0.0031 \times PaO_2 \ (mmHg).$$

The hemoglobin concentration was measured by using arterial blood with a hemacytometer for animals (pocH-100iV Diff, Sysmex Corporation, Kobe, Japan).

We measured blood gas parameters and the hemoglobin concentration 1 time per sample.

GPx activity: Approximately 2.0 ml venous blood collected via a catheter was placed into a tube containing sodium heparin and centrifuged at $1,500 \times g$ for 10 min at 4.0°C. The plasma layer and buffy coat were removed to obtain erythrocytes. The erythrocytes were lysed in 4 volumes of ice-cold

high-performance liquid chromatography (HPLC) grade water and centrifuged at $10,000 \times g$ for 15 min at 4.0°C because hemoglobin absorbs significantly at 340 nm, and thus erythrocyte lysates must be diluted before assaying [38]. The supernatant was collected and frozen until analyzed for GPx activity using a commercial GPx assay kit (GPx Assay Kit, Cayman Chemical Co., Ann Arbor, MI, U.S.A.). This kit measures GPx activity using a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPx, is recycled to its reduced state by GR and nicotinamide adenine dinucleotide phosphate (NADPH).

$$R\text{-}O\text{-}O\text{-}H + 2 \text{ glutathione} \xrightarrow{\text{GPx}} R\text{-}O\text{-}H + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{GR}} 2 \text{ glutathione} + NADP^+$$

The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the Δ_{340} is directly proportional to the GPx activity in the sample. The GPx activity assay range of this kit was between 50–344 nmol/min/ml. We measured GPx activity 3 times per sample.

Power spectral analysis of heart rate variability: LF/HF power values and RRI reflect autonomic nervous activity [1, 7, 40]. A small electrocardiograph (Digital Quick Corder QR2500, Fukuda M-E Kogyo Co., Ltd., Tokyo, Japan), suitable for small animals was used for electrocardiogram (ECG) recording. Electrodes were taped to the chests of the dogs, and the dogs wore a vest with the electrocardiograph in its pocket [22]. The data were collected every 24 hr, and LF/HF and RRI were calculated for every 5 min using an HS1000 Lite Holter analysis system (Fukuda M-E Kogyo Co., Ltd.).

LF and HF power analysis was performed by power spectral analysis using the fast Fourier transform method. Briefly, 100 sec blocks of the date were resampled at 1.28 samples/sec and subjected to a Hamming window [14]. If there was a run of arrhythmia or an artifact longer than 1 beat in length, the partial block was discarded, and a new block was started as the first of the 100 sec blocks. The frequency range of the power spectra was 0.01 to 0.64 Hz. LF power was defined as the energy in the power spectrum between 0.04 and 0.15 Hz. HF power was defined as the energy in the power spectrum between 0.15 and 0.40 Hz. LF/HF was defined as the ratio of LF power to HF power.

Beat-by-beat RRI data were obtained from the beat stream file. A linearly interpolated beat was substituted to exclude intervals of ectopy or artifacts less than or equal to 2 RRI [46].

We used the following time points for the dogs: (1) quiet, a 5 min time point when the dogs were in their cages before the start of the study; (2) running, 5 min after running; (3) non L-HBOT, 5 min after non L-HBOT; and (4) L-HBOT, 5 min after L-HBOT.

Measurement of stress-related hormones: All stress-related hormone levels were measured by an external facility (Japan Clinical Laboratories Inc., Osaka, Japan). Approximately 7.0 ml of venous blood was collected from the catheter. Of this, approximately 2.0 ml was placed into a tube with serum separating medium and centrifuged at $1,000 \times g$ for 10 min at 4.0°C. The serum layer was removed and frozen at −80°C until analyzed for cortisol levels using an electrochemiluminescence immunoassay. Briefly, samples were added to beads coated with an anti-cortisol antibody, and then an antiprothrombotic antibody was added that labeled the ruthenium complex. This ruthenium complex emitted light as a result of an electrochemical change. In this way, we could measure the level of cortisol in the samples. The assay range of cortisol was from 0.1 to 400 μg/ml.

The remaining 5.0 ml of collected blood was injected into a tube containing sodium EDTA and centrifuged at $1,000 \times g$ for 10 min at 4.0°C. The plasma layer was removed and frozen at −80°C until analyzed for adrenaline and noradrenaline levels using HPLC. Briefly, the test material in the liquid mobile phase, which was in contact with the stationary phase, was isolated by the gap of the affinity to both phases. The fraction isolated with the test material was identified and quantitatively determined by its chromatogram acquired with a detector. The assay ranges of both adrenaline and noradrenaline were 6 to 10^7 pg/ml.

SC: SC was measured using a measuring instrument for dogs (PS-IMP001, LiveAid Co., Ltd.). Electrodes (Echorode III, Fukuda Denshi Co., Ltd., Tokyo, Japan) were attached to the metacarpal pads, which contain sweat glands, and a pulse of 3.0 volts was applied. When sympathetic nervous activity increases, sweat glands produce more sweat, and skin impedance decreases, resulting in increased SC. We measured percentage SC versus the conductance without impedance calculated by the instrument [18]. We measured SC 3 times per treatment.

Statistical analysis: The results were analyzed by parametric methods, using Statcel 3 (OMS Publishing, Tokorozawa, Japan), and mean and standard deviation (SD) values were reported. Differences for (1) quiet, (2) running, (3) non L-HBOT and (4) L-HBOT in each of the measurement indicators were tested by analyses of variance and Dunnett's tests. Values of $P<0.05$ were considered significant in all analyses.

RESULTS

Appearance of the dogs: Each dog walked around and smelled the chamber for the first 5 to 10 min of L-HBOT or non L-HBOT. After that, the dogs lay down calmly. None of the dogs showed any symptoms, such as seizures, salivation or vomiting, after L-HBOT.

Blood gas parameters: The hemoglobin concentration of the 4 dogs was 17.10 ± 1.3 g/dl, almost within the reference range of 12.0 to 18.0 g/dl. None of the hemoglobin concentrations were significantly changed after L-HBOT (the results are not shown). There were no significant differences in any blood gas parameters among the dogs when they were quiet, after running, after non L-HBOT or after

Table 1.   Blood gas parameters for the 4 treatments

Treatment	PaO_2 (mmHg)	SaO_2 (%)	CaO_2 (ml/dl)	pH	$PaCO_2$ (mmHg)
(1)	86.0 ± 4.3	94.0 ± 4.2	21.9 ± 0.7	7.25 ± 0.23	26.7 ± 8.6
(2)	132 ± 55.9	99.0 ± 1.4	23.2 ± 0.8	7.39 ± 0.05	24.9 ± 8.9
(3)	91.5 ± 16.3	96.5 ± 0.7	26.5 ± 0.1	7.35 ± 0.05	33.9 ± 4.3
(4)	90.0 ± 7.1	97.5 ± 0.7	22.7 ± 0.1	7.40 ± 0.00	23.6 ± 5.1

The mean ± SD values for PaO_2, SaO_2, CaO_2, pH and $PaCO_2$ of arterial blood of the 4 dogs (1) when they were quiet, (2) after running, (3) after non L-HBOT and (4) after L-HBOT.
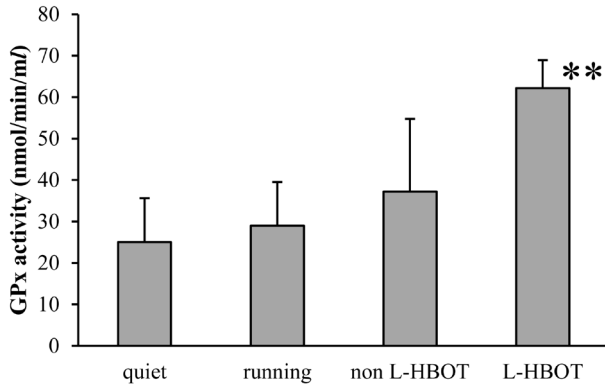

Fig. 2.   GPx activity for the 4 treatments. GPx activity in erythrocytes of the 4 dogs when they were quiet, after running, after non L-HBOT and after L-HBOT. The bars indicate mean values, and the lines indicate the SD. **Significant difference vs quiet (*P*<0.01).
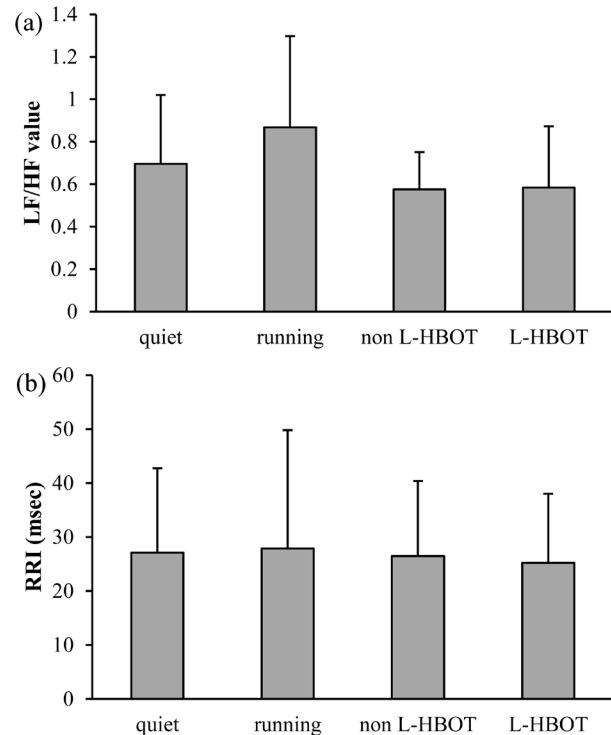

Fig. 3.   Power spectral analysis of heart rate variability for the 4 treatments. Power spectral analysis of heart rate variability of the 4 dogs when they were quiet, after running, after non L-HBOT and after L-HBOT. (a) LF/HF value. (b) RRI. The bars indicate mean values, and the lines indicate the SD.

L-HBOT. PaO_2 and SaO_2 values were within the reference ranges (PaO_2, ≥80.0 mmHg; SaO_2, 95 to 100%) [11, 51]. The CaO_2 value was within the range calculated by the equation stated above with the PaO_2 and SaO_2 reference range (16.0 to 25.0 ml/dl). Although pH was low (when the dogs were quiet) or almost normal (after the running and L-HBOT conditions), hypocapnia was observed, except for after non L-HBOT (the reference ranges: pH, 7.35 to 7.45; $PaCO_2$, 33.0 to 45.0 mmHg) (Table 1) [51].

GPx activity: The GPx activity in erythrocytes of each dog when they were quiet was 25.0 ± 10.6 nmol/min/ml. After running, it was 29.0 ± 10.5 nmol/min/ml; after non L-HBOT, it was 37.2 ± 17.6 nmol/min/ml; and after L-HBOT, it was 62.2 ± 6.8 nmol/min/ml. The mean GPx activity after L-HBOT was about twice as high as that when the dogs were quiet (*P*<0.01) (Fig. 2). The GPx activity after running tended to decrease compared with that when the dogs were quiet, but this was not statistically significant. Some of the GPx activity values were out of the assay range, because lower values were measured in the erythrocyte samples due to the interference of the absorbance of hemoglobin, as indicated in the resources provided with the assay kit and a previous report [38].

Power spectral analysis of heart rate variability: One male dog was excluded from the analysis, because he had removed the vest that contained the electrocardiograph. Therefore, we analyzed the data of 3 dogs. The LF/HF value

of each dog when they were quiet was 0.69 ± 0.32. After running, it was 0.87 ± 0.43; after non L-HBOT, it was 0.58 ± 0.18; and after L-HBOT, it was 0.58 ± 0.29. RRI when the dogs were quiet was 27.1 ± 15.7 msec. After running, it was 27.9 ± 21.9 msec; after non L-HBOT, it was 26.5 ± 13.8 msec; and after L-HBOT, it was 25.2 ± 12.8 msec. There were no significant differences in the mean LF/HF and RRI between the quiet, after running, after non L-HBOT and after L-HBOT conditions (Fig. 3).

Stress-related hormone levels: The cortisol level of each dog when they were quiet was 7.1 ± 1.9 µg/dl. After running, it was 8.3 ± 3.3 µg/dl; after non L-HBOT, it was 3.9 ± 2.1 µg/dl; and after L-HBOT, it was 3.0 ± 1.1 µg/dl. The adrenaline level when the dogs were quiet was 30.0 ± 21.8 ng/ml. After running, it was 16.5 ± 12.3 ng/ml; after
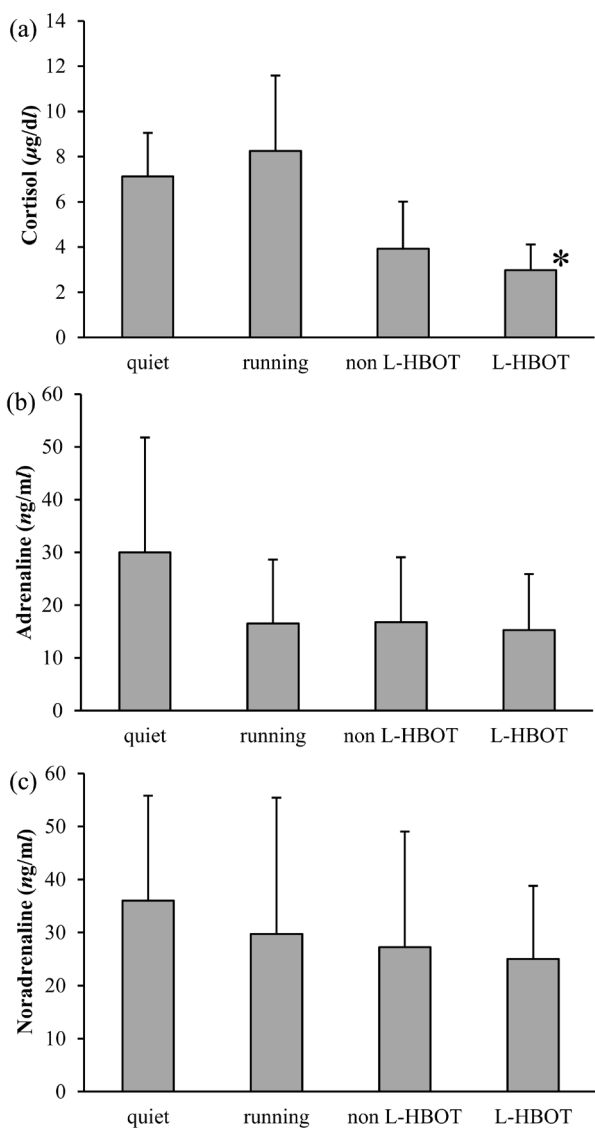
(a)

(b)

(c)

Fig. 4. Stress-related hormone levels for the 4 treatments. Stress-related hormone levels in blood of the 4 dogs when they were quiet, after running, after non L-HBOT and after L-HBOT. (a) Cortisol levels. (b) Adrenaline levels. (c) Noradrenaline levels. The bars indicate mean values, and the lines indicate the SD. *Significant difference vs quiet ($P<0.05$).

non L-HBOT, it was 16.8 ± 12.3 ng/ml; and after L-HBOT, it was 15.3 ± 10.7 ng/ml. The noradrenaline level when the dogs were quiet was 36.0 ± 19.8 ng/ml. After running, it was 29.8 ± 25.7 ng/ml; after non L-HBOT, it was 27.3 ± 21.8 ng/ml; and after L-HBOT, it was 25.0 ± 13.8 ng/ml. The cortisol level after L-HBOT was significantly lower than that when the dogs were quiet ($P<0.05$). There were no significant differences in the mean adrenaline or noradrenaline among the treatments (Fig. 4).

SC: The SC of each dog when they were quiet was 12.6 ± 6.5%. After running, it was 47.8 ± 40.8%; after non L-
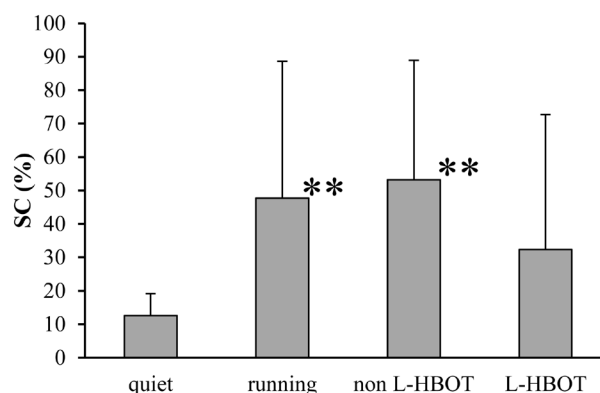

Fig. 5. SC for the 4 treatments. SC of the 4 dogs when they were quiet, after running, after non L-HBOT and after L-HBOT. The bars indicate mean values, and the lines indicate the SD. **Significant difference vs quiet ($P<0.01$).

HBOT, it was 53.2 ± 35.7%; and after L-HBOT, it was 53.2 ± 35.7%. The mean SC was significantly higher after running ($P<0.01$) and after non L-HBOT ($P<0.01$) than when the dogs were quiet (Fig. 5). There was no significant difference in mean SC between when the dogs were quiet and after L-HBOT.

DISCUSSION

In the present study, blood gas parameters were not significantly changed after exercise or L-HBOT. A study that included healthy Labrador Retrievers indicated that immediately after 10 min of repeatedly retrieving a soft plastic tube thrown approximately 40 to 50 yards on land, the PaO_2 of the dogs was significantly increased to 140.3 ± 17.8 mmHg [30]. That report suggested that hyperventilation was induced response to increased oxygen demand after strenuous exercise and caused an increase in PaO_2. On the other hand, a study with obese dogs indicated that the blood oxygen saturation based on pulse oximetry of the dogs after walking for 6 min at their own pace decreased significantly compared with that of dogs that participated in a weight loss program [28]. As compared with these previous studies, the exercise load might not have been large enough to change the blood gas parameters in the present study. The pH level increases, and $PaCO_2$ decreases in hyperventilation [9, 28, 30]. In the present study, although the pH level and $PaCO_2$ were low especially when the dogs were quiet, these two parameters did not significantly change after L-HBOT. From these results, L-HBOT may be useful in improving the oxygenation dynamics without inducing hyperventilation.

H-HBOT sometimes induces hyperoxia, resulting in central nervous or pulmonary oxygen toxicity [21]. A previous study showed that newborn dogs that received H-HBOT with 100% oxygen pressurized at 5.0 ATA for about 40 min experienced seizures [49]. This very high oxygen concentration and pressure induces the oxidation of mitochondrial nicotinamide adenine dinucleotide, which results in seizures.

In the present study, we did not see seizures or other neurological symptoms, and although the PaO_2 after L-HBOT was lower than the value we had expected, the dogs did not show hypoxia or any pulmonary oxygen toxicity symptoms, such as chest pain or dry cough. So, we considered that L-HBOT may not induce central nervous or pulmonary oxygen toxicity. Low PaO_2 after L-HBOT may be caused by problems related to measurement; the sampling position or delay of measurements may influence the values [17].

An important finding of the present study was that GPx activity increased after L-HBOT compared with after running in the dogs. This result confirmed that the L-HBOT could increase GPx activity and reduce ROS generated by stressful events. In a previous study in rats, GPx activity increased in lung tissue and erythrocytes up to 30 min after H-HBOT with 100% oxygen pressurized at 3.0 ATA [3]. Moreover, another previous study recognized that GPx activity was higher in erythrocytes than in other tissues in rats [29]. Although only a few *in vivo* studies have investigated GPx activities in dogs under HBOT, we have shown that GPx activity in erythrocytes increased at 30 min after L-HBOT with 30% oxygen pressurized at 1.3 ATA in dogs. On the other hand, prolonged HBOT (7 to 15 HBOT sessions, one session/day) may induce increased levels of ROS in the blood [6, 34]. It is thought that activation of the redox-sensitive transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2), may play a pivotal role in the cellular defense against oxidative stress via transcriptional upregulation of phase II defense enzymes and antioxidant stress proteins, such as GPx; however, there have been only a few reports on this [2]. We considered from our results that the generation of low levels of ROS following a small increase in the supply of oxygen may activate Nrf2 and result in increased GPx activity. Further studies are required to determine the relationship between L-HBOT and the generation of ROS, Nrf2 and GPx.

We have previously shown that SC reflects the sympathetic nervous activity in dogs [18]. In the present study, SC and the cortisol level decreased after L-HBOT compared with after running in the dogs, indicating that L-HBOT may control the sympathetic nervous activity in dogs. In professional divers, the heart rate and LF/HF values decreased during H-HBOT with 100% oxygen at 2.5 ATA for 60 min [27]. This result suggests that H-HBOT may control sympathetic nervous activity and increase parasympathetic nervous activity. Increased peripheral vessel resistance as a result of H-HBOT may increase vagal efferent discharge. This would result in increased parasympathetic nervous activity and a decreased LF/HF value. L-HBOT may cause the same physiologic responses to happen.

The reactions of SC and stress-related hormones or the heart rate were verified in previous studies in human beings; however, the results varied. For example, SC and adrenaline levels were correlated with perioperative stress, but the heart rate was not [43]. Another study showed that both stress-related hormones and the heart rate did not significantly reflect the stress level [24]. Our previous study confirmed similar reactions for SC and stress-related hormones in dogs during the perioperative period [18]. There are only a few

reports available on SC, stress-related hormones and heart rate during HBOT in humans or other animals [26], so further experiments are required to verify these relationships.

This study has some limitations. We studied only a small numbers of dogs, and all of them were beagles. L-HBOT should be applied with caution concerning the respiration state in some kinds of dogs, especially brachycephalic dogs in which respiratory diseases often occur [35]. Moreover, increased red blood cells, hemoglobin and hematocrit are observed in sight hound dogs [47]. The oxygen circulation in these dogs is considered to be different from that of the dogs included in this study. More studies of L-HBOT with more blood gas parameter samples and other kinds of dogs are needed to create more adequate protocols and clarify the safety.

In the present study, we provided important evidence for L-HBOT in dogs. L-HBOT has low influences on blood gas parameters in dogs. On the other hand, the increased GPx activity after L-HBOT may bring new insights regarding oxidative stress mechanisms. Moreover, L-HBOT may restrain autonomic nervous activity. L-HBOT may also change the oxidative stress mechanism and autonomic nervous activity and therefore control body homeostasis.

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